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ORIGINAL ARTICLE

False Human Immunodeficiency Virus Test Results Associated with Rheumatoid Factors in Rheumatoid Arthritis[△]

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Key words: human immunodeficiency virus; false positive; rheumatoid arthritis; antibody

Objective To investigate if immunological factors associated with rheumatoid arthritis (RA) affect the result of human immunodeficiency virus (HIV) screening by electrochemiluminescence immunoassay (ECLIA) and enzyme-linked immunosorbent assay (ELISA).

Methods 100 RA cases were enrolled from January 2012 to February 2013 into this study. HIV screening was conducted with ECLIA detecting both HIV-1 p24 antigen, HIV-1 and HIV-2 antibodies, with ELISA and colloidal gold method detecting HIV-1 and HIV-2 antibodies. The samples producing positive results were submitted to the Center for Disease Control for confirmation using Western blotting method. The antibody titers of rheumatoid factors (RF) including RF-IgG, RF-IgM, RF-IgA, and CCP-IgG were analyzed by ELISA.

Results The HIV positive-rate determined by ECLIA was significantly higher than that by ELISA and colloidal gold method ($P < 0.01$). The false-positive rate of HIV screening was associated with antibody titers of RF-IgG, RF-IgM, RF-IgA, and CCP-IgG in RA ($P < 0.01$).

Conclusion Immunological factors, including RF and anti-CCP antibody, may influence the screening of HIV by ECLIA, producing false-positive result.

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HUMAN immunodeficiency virus (HIV) remains one of the most serious challenges to health worldwide. Approximately half of the infected people were under the age of 25.^{1,2} Immunoassay-based serological tests for HIV have enabled simple and

rapid detection of infection and have been widely implemented as screening tools for both diagnosis and epidemiological monitoring.² In this study, we attempted to identify the immunological factors associated with the false-positive HIV screening results in rheumatoid arthritis (RA).

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PATIENTS AND METHODS

Patient selection

100 RA patients were selected randomly between January 2012 and February 2013, aged 32 to 67 years, including 79

females and 21 males. The patients completed questionnaire and clinical data. The mean age of the patients was 49.5 ± 16.5 years. Rheumatoid factors (RF) and anti-CCP antibody titers were determined using enzyme-linked immunosorbent assay (ELISA) kits (AESKU. DIAGNOSTICS, Wendelsheim, Germany). According to their immunological factors titers, RA cases enrolled into this study were classified into two groups: low titer group (RF-IgA, RF-IgG, and RF-IgM <18 U/ml, anti-CCP <25 U/ml), and high titer group (RF-IgA, RF-IgG, and RF-IgM >300 U/ml, anti-CCP >500 U/ml).

HIV screening methods

HIV screening was performed with electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics GmbH, Penzberg, Germany) detecting both HIV-1 p24 antigen, HIV-1 and HIV-2 antibodies, and ELISA (Wantai, Beijing) and colloidal gold method (SD Biosensor, Suwon-si, Korea) detecting only HIV-1 and HIV-2 antibodies. The specimens with positive results were sent to the Center for Disease Control for confirmation by Western blotting. False-positive was considered if screening results were positive and the confirmation results were negative. The patients were interviewed about their sexual behavior and assessed based on signs and symptoms of sexually transmitted infections.

Statistical analysis

The analysis was restricted to individuals of whom complete clinical data were available. Data were processed using SPSS 13.0 software. Categorical data were expressed

as frequencies, and compared using χ^2 test. $P < 0.05$ was considered statistically significant.

RESULTS

Positive rates detected by ECLIA and ELISA

The positive rate of HIV screening by ECLIA was higher than that by ELISA and colloidal gold method in RA (16% versus 0%, $P < 0.01$). 16 of the 100 samples were positive as determined by ECLIA, but ELISA and colloidal gold method presented negative results. The 16 ECLIA positive samples were examined with Western blotting. 13 of which had unconfirmed results. The 13 samples were re-tested after one month, 10 of which produced negative results, and the other 3 were still given unconfirmed results. Three months later, the 3 samples presented negative results as well. The 16 cases all had false-positive results as confirmed by Western blotting.

Association of immunological factors titers with HIV false-positivity in RA

The incidences of positive HIV result determined by ECLIA in different immunological factors groups were 2.7% (1/37) in RF-IgM <18 U/ml group, 32.4% (11/34) and 13.6% (3/22) in RF-IgM >300 U/ml and RF-IgG >300 U/ml group (Table 1). Meanwhile, the positive rate in anti-CCP <25 U/ml and >500 U/ml groups were 4.7% (2/43) and 24.6% (14/57) (Table 2). The false-positive rate of HIV screening was associated with antibody titers of RF-IgG, RF-IgM, RF-IgA and CCP-IgG in RA ($P < 0.01$).

Table 1. Human immunodeficiency virus (HIV) testing results by electrochemiluminescence immunoassay in groups stratified by rheumatoid factor (RF) titer [n (%)]

HIV screening result	RF-Ig titers <18 U/ml			RF-Ig titers >300 U/ml			Total
	IgM	IgG	IgA	IgM	IgG	IgA	
Positive	1 (2.7)	0	0	11 (32.4)*	3 (13.6)*	0	16
Negative	36 (97.3)	0	0	23 (67.6)	19 (86.4)	7 (100.0)	84
Total	37			34	22	7	100

* $P < 0.01$ compared with RF <18 U/ml HIV positive group.

Table 2. HIV testing results by electrochemiluminescence immunoassay in groups stratified by anti-CCP titer [n (%)]

HIV screening result	Anti-CCP titer		Total
	<25 U/ml	>500 U/ml	
Positive	2 (4.7)	14 (24.6)*	16
Negative	41 (95.3)	43 (75.4)	84
Total	43	57	100

* $P < 0.01$ compared with anti-CCP <25 U/ml HIV positive group.

DISCUSSION

Testing for HIV was begun in 1985 with the introduction of the enzyme immunoassay. Different screening methods including ELISA, ECLIA, and colloidal gold method have been developed, having varying sensitivity and specificity.^{3,4}

ECLIA detects both HIV-1 p24 antigen, HIV-1 and HIV-2 antibodies, while ELISA and colloidal gold method detect only HIV-1 and HIV-2 antibodies.^{4,5} In this study, the positive rate of HIV tests by ECLIA was higher than that by ELISA and colloidal gold method in RA. In the 16 cases with positive results submitted for confirmation by the Center for Disease Control using Western blotting, 13 were uncertain, of whom 10 had negative results in the reexamination one month later, and the other 3 were negative after 3 months by confirmatory testing. Furthermore, among the RA patients, we observed an association between false-positive results and specific immune factors, especially increased titers of RF-IgG, RF-IgM, and anti-CCP antibody. The false-positive rates in RF-IgG<18 U/ml group, RF-IgM<18 U/ml group, and anti-CCP<25 U/ml group were significantly lower than those in RF-IgG>300 U/ml group, RF-IgM>300 U/ml group, and anti-CCP>500 U/ml group.

RF is an antibody against the Fc portion of immunoglobulin G and is associated with autoimmune diseases, such as RA. However, it can also be produced in individuals with viral, bacterial, and other parasitic infections.^{5,6} RA patients often produce auto-antibodies against proteins and peptides containing citrulline. Citrulline is generated in an inflammatory environment through modification of arginine by peptidylarginine deiminase. Anti-CCP antibody is found in serum up to 10 years before the onset of joint symptoms in patients who later develop RA and may appear somewhat earlier than RF.⁷ A positive anti-CCP antibody test result is more consistent with early or even preclinical RA, since this test, unlike RF testing, is generally negative in the setting of infection. We speculated that the mechanisms of the reactivity with HIV p-24 was antigenic mimicry between self-epitopes, such as anti-CCP antibody and the RF-Fc portion of immunoglobulin G.

This study investigated the possible mechanisms of false positivity observed in HIV screening with ECLIA in RA patients. While the rate of false-positive results is of considerable concern, we believe that this problem is not restricted to ECLIA. Further research on the cross-reactivity of HIV diagnostic tests with RA is warranted. Several studies have suggested that ECLIA assay is both highly sensitive and specific.⁸⁻¹⁰ From the study we can also deduce that

ECLIA detecting is more sensitive than ELISA and colloidal gold method with rheumatoid samples. While specificity should be improved when tests are applied in combination, the high rate of false-positivity associated with ECLIA assay may lead to a considerable rise in the cost of testing, since positive test results by a particular assay require confirmation when that assay is used as part of a testing method.

This study had several limitations. First of all, it was difficult to disentangle the independent effects of each immunological factor. Second of all, this was a cross-sectional analysis, so we cannot determine the causality of the associations observed. A large proportion of the immunological factors were associated with false-positive results, and the causes of false-positive HIV screening result may vary with methods. In addition, this study cannot clarify if the same factors of false-positive ECLIA result apply to other HIV tests. Further studies are needed to confirm our findings, elucidate the immunology mechanisms for increased HIV false-positive results screened by ECLIA in RA patients.

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